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New British and American species of Lobomonas: a study in morphogenesis of motile algae

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(WITH PLATES 5 AND 6)

The genus *Lobomonas* was established in 1899 by Dangeard (**3**) on a single species, *L. Francei*, found somewhat frequently in the vicinity of Poitiers, France. This species had been figured twenty-one years previously by Stein (**7**, *pl. 13, f. 17, 18*) as a form of *Chlamydomonas pulvisculus* Ehrenb. Golenkin also appears to have had this species in Russia, and to have confused it with another genus, for one of his figures (**5**, *f. 19*), described as a reduced form of *Pteromonas alata* (Cohn) Seligo, can hardly be anything else than a young cell of *L. Francei*. Apparently the species has not been studied or scarcely even reported otherwise, except by Dangeard. In 1902 Chodat (**2**) transferred to the newly founded genus his species *Chlamydomonas stellata*, briefly described six years before (**1**); he even appears to have been doubtful of the distinctness of his form from Dangeard's type, though both species have been accepted by West (**8**, p. 172) and Wille (**9**, p. 19), who have reproduced the original illustrations.

The genus presents a cell organization almost precisely like that of *Chlamydomonas*, probably its nearest relative, with the exception that its outer wall is furnished with variable irregularities or protuberances, which in the type species are frequently more developed on the posterior part of the cell, while in *L. stellata* the more uniformly triangular lobes are figured as covering the wall nearly to the region of insertion of the cilia. It

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is probable that *Lobomonas* has escaped the notice of collectors to some extent because of the minute size of the cells, but doubtless it is actually one of the rarest genera of the *Chlamydomonas* group. The two new species now presented serve to emphasize the unity and distinctness of the genus, and also provide certain features of division and conjugation not known hitherto, which indicate a fundamental parallelism with *Chlamydomonas*.

Lobomonas pentagonia sp. nov.

This species, the first of the genus to be reported in England, I believe, was discovered in considerable abundance, together with two species of *Pteromonas*, at Ham Common, Surrey, near Kew, in 1920. The habitat was the border of a small shallow pond at the west end of the common, a place frequented by cows and horses, and therefore supplied with water containing a considerable amount of nitrogenous organic matter in solution: in fact the general conditions were very like those of the much smaller pool where *Lobomonas rostrata*, to be described presently, was found with another species of *Pteromonas* in New Jersey.

Like all other species of the genus, *L. pentagonia* is very minute, but it is more constant in form than *L. Francei* or *L. rostrata*. When seen in side view the cell appears rather pentagonal in outline (FIGS. 1–4), but careful focussing shows that the angles do not all lie in the same plane. Polar views often show five to eight protuberances (FIG. 14), likewise not all at the same level. One of the most symmetrical specimens, when resting with its ciliated end turned upward, shows four anterior protuberances and, alternating with them, four others at the posterior end of the zoospore (FIG. 5); the number, however, is variable. The protoplast, including the chromatophore, in young individuals usually extends into and fills the protuberances, and ends anteriorly in a somewhat obtuse beak, to which the two cilia are attached (FIG. 1). In older specimens the protoplast usually retracts from one or more of the wall protuberances and leaves them empty: they do not appear like dense gelatinous structures. The chromatophore is more or less hollowed out in the common *Chlamydomonas* fashion but thickened in the region where the pyrenoid lies in a somewhat lateral position. The two alternately pulsating vacuoles lie in a plane nearly perpendicular to that passing through the two extended cilia (FIG. 5), as in most species of *Chlamydomonas*, so that in the ordinary face view of the cell only one is usually seen. The

narrow rod-shaped red eye-spot lies in or just beneath the plasma membrane slightly in front of the middle of the cell. Its position with relation to the cilia is more like that which it occupies in *Brachiomonas* than that in *Chlamydomonas*, as brought out recently by the writer (**6**), though the dorsiventral differentiation here is perhaps less constant and definite than in the two related genera.

ASEXUAL REPRODUCTION. Dangeard (**3**) reports that his attempts to cultivate *L. Francei* in a moist chamber did not succeed, and that it was difficult to obtain the multiplication of the organism. He shows that the cells come to rest and generally become rounded in shape, then divide into four or eight daughter cells which escape as zoospores. He gives no figure to support Wille's (**8**) surmise that division is longitudinal. In our two new species I found that very generally after motile cells were mounted in a hanging drop they would for the most part come to rest in a few hours and proceed to divide, though in many cases the daughter cells failed to become motile or escape. In both species pyrenoids were not seen in any dividing specimens until the daughter cells had begun to take on the typical form, so that it would appear necessary to conclude that in this genus, as in *Brachiomonas* and in some species of *Chlamydomonas*, the pyrenoid disappears before the first cleavage, and that one is formed *de novo* in each daughter cell.

In *L. pentagonia* the first division plane usually appears at first sight to be transverse to the longitudinal axis of the cell, but several cases were observed which lead to the conviction that there is regularly a rotation of the protoplast during or before the beginning of cleavage. FIGS. 6 and 7 show two stages of division where the protoplast has revolved to an oblique position when the cleavage begins; probably the rotation was delayed more than usual in this case; here the original contractile vacuoles persist after the division of the nucleus. Fig. 8 shows a case where what I am sure the original vacuoles persisted until the end of cleavage into two daughter cells. In case of the formation of four zoospores the second plane of cleavage is perpendicular to the first, *i. e.* it lies in a longitudinal axis of the mother cell: it may lie in a single plane through the two halves (FIG. 11), or the second division plane in one half cell may be perpendicular to that in the other (FIG. 12).

SEXUAL REPRODUCTION apparently has not been observed hitherto in this genus. I considered it good fortune, therefore, to discover three or four cases of conjugation taking place almost simultaneously in one hanging drop. The gametes have the general form and character of the vegetative cells, but are much smaller, provided with an excessively delicate cell wall, and the cilia are longer than the cell body. In the first case seen, one gamete was rather broad and pillow-shaped, with a distinctly visible cell wall, while the other was narrower and probably more nearly cylindrical, and furnished with a wall so delicate that it was detected only in its subsequent behavior. The two gametes became engaged by their cilia, but not otherwise in contact, and remained in this position for at least a half hour, with slight dancing movement (FIG. 15); the cilia, most if not all of this time, were trailed backward along the sides of the narrower gamete. The first movement toward joining was in a sudden break of the anterior papilla of the broad gamete, whereby a broader papilla of colorless cytoplasm surged forward and presently plastered itself on the beak of the unchanged gamete (FIG. 16), the remainder of the protoplast of the broader gamete then distinctly withdrawing from the posterior part of its wall (FIG. 17). Now for a period of about a half hour the remainder of the protoplast of the broader gamete was gradually oozing out of its wall and into the narrower gamete: even yet the narrower gamete hardly showed a wall, but it seemed evident that one must be present, since the posterior part of this gamete rigidly retained its original form (FIGS. 19, 20). After this point more active ciliary movement carried the zygote beyond possibility of observation.

A second case showed a similar figure of the narrow gamete remaining rigid for as long a time as it could be followed. In all probability this wall of the narrow gamete is finally thrown off separately, permitting the rounding up of the plasmatic mass to form a spherical zygospore. A third case showed the gametes more nearly equal, and it was clear that the walls of both were practically alike in character and not easily abandoned (FIG. 21), so that fusion was long delayed.

There is in this species apparently little differentiation between the gametes; indeed one might be inclined to regard the difference in size as merely accidental. Nevertheless from the behavior of the cilia, that is, both pairs for the most of the time stretching back alongside the narrower gamete, one may assume

a certain degree of differentiation. The conjugation of this species presents a close parallel to that described by Goroschankin for *Chlamydomonas reticulata* (**5**, p. 126, *pl. 3, f. 6–8*). He states that the gametes show little difference in size, and that they sometimes simultaneously slip out of their walls; but more frequently, after the beginning of conjugation, one of the gametes first throws off its wall and takes on a globose form, then the second does likewise, and then the two rounded masses go on to complete fusion.

The subjoined Latin diagnosis presents the chief characteristics of this species:

Lobomonas pentagonia sp. nov. *L.* cellulis vegetativis minutis, membrana a latere aspectata forma aliquanto pentagonia sed angulis rotundatis (verrucis) haud omnibus in eodem plano a vertice aspectata rotundata cum 5–8 verrucis, aliis anterioribus, aliis posterioribus protoplasto vel membranae conformali vel plus minus contracto et ellipsoideo, cum rostello conico ad quod cilia bina cellulae longitudinem fere adequantia affiguntur; chromatophoro excavato, pyrenoidem unum sublateralem portante, et in parte excavata nucleum lateralem includente; stigmate bacilliformi paululum ante mediam cellulam sito; vacuolis contractilibus binis in rostello cytoplasmatico positis.

Propagatio fit 2 aut 4 zsooporis intra cellulae matricalis membranam ortis, divisione priore visa quasi transversaria propter protoplasti rotationem sed vero longitudinali.

Generatio fit gametis parvulis, vel subaequalibus vel aliquanto disparibus, membrana tenuissima vestitis, inter se binatim copulantibus.

Longit. cell. veg. 10–13 μ; lat. 9–10 μ. Longit. gametarum ca. 8 μ; lat. 4–5 μ; longit. ciliorum ca. 13 μ.

Hab. in stagni margine. Ham Common, Surrey, England, 1–7 Aug. 1920.

Lobomonas rostrata sp. nov.

This form, the first representative of the genus to be reported in America, at first sight appeared very similar to *L. Francei* Dangeard (**3**, p. 115), but careful study disclosed differences as important as those which distinguish most species of *Chlamydomonas*, so that I feel obliged to regard it as a new species. I first found a few individuals in examining a collection of *Gonium pectorale* and an undetermined species of *Chlamydomonas*, obtained the last of September, 1919, from a rain-water pool of a highway in the southern part of Englewood, New Jersey. Later it was interesting to discover that the species had been collect-

ed about a week earlier in a much deeper pool, about half a mile distant from that just mentioned; this discovery was due to the fact that a fine colony of the *Lobomonas* developed on an agar plate containing a sample of my first collection of *Pteromonas* from this pool. The new species continued to appear sporadically in later gatherings from the same pool, the last being made November 12; it was also collected in October, together with *Chlamydomonas metastigma* Stein, from another rain-water pool in a wheel-rut, not far from the one first mentioned, though separated from it by railroad tracks bordered by a deep ditch on either side. The first mentioned wheel-rut yielded a few individuals in the following season. A few specimens of this species were also discovered during the past summer in a collection from a similar wheel-rut in northern Vermont: here it was accompanied by *Gonium pectorale*, *Pandorina*, *Chlorogonium*, *Chlamydomonas*, and a very interesting new form of *Polyblepharides*, to be described in a forthcoming paper. The *Lobomonas* never appeared to be abundant like its associates in the same pools; usually not more than a dozen or two specimens turned up in one hanging drop mount.

The vegetative cells or zoospores of this species most commonly have a somewhat obpyriform shape (FIG. 22–27) though they are sometimes almost ellipsoid. In younger individuals the cell wall is so delicate and close-lying as to be indistinguishable for the most part, but in older cells it is well developed (FIGS. 28, 29); it is generally produced into a variable number of lobes, of which from five to seven or sometimes as many as ten appear in a face view; that these lobes are developed on all sides of the cell is clearly shown in a polar view (FIG. 30). At the anterior, usually broader end, the wall is extended into a truncate, wedge-shaped beak, or possibly more typically this takes the form of a more or less double papilla (FIG. 33); on account of the minute size of the organism it is often most difficult to see clearly the exact structure of this protuberance, which is one of the most characteristic features distinguishing this species from the two hitherto described in Europe. Sometimes the wall appears to be uniformly thin, sometimes thickened at the end of the lobes, and sometimes considerably thickened throughout. The protoplast, indistinguishable outwardly from the bright green chromatophore, fills the lobes in young individuals; in older cells it retracts more or less, so as to leave some or all of the lobes colorless. In such cases it is not easy to determine whether the

lobe is a dense, gelatinous structure, or simply membranous and separated by a space from the protoplast. In certain cases the latter interpretation is clearly indicated, for the tip of the lobe is manifestly of thickened grayish wall substance, with a clear space inside (FIG. 37). The cilia are attached at a single point to the anterior end of the protoplast, which is usually obtuse, though it may have a slight beak; they immediately diverge at a wide angle to pass separately through the papilla of the wall, and are often seen in quiescent individuals stretching out stiffly in the form of a v; their length is as variable as the cell outline, often being less than the cell length, but perhaps more characteristically distinctly greater than the cell length. At the base of the cilia are the alternately pulsating vacuoles, lying regularly in such a position that a line passing through the two is perpendicular to the plane in which the quiescent cilia lie, so that only one of the vacuoles is seen when both cilia are equally clear, but both may appear at the same level when one of the cilia is behind the other (FIG. 29). The single pyrenoid occupies a lateral position in the deeply hollowed out chromatophore (FIGS. 24, 26), contrasting sharply with the axial pyrenoid in a massive chromatophore described and figured by Dangeard (3) in *Lobomonas Francei*. The small rod-shaped red eye-spot lies in front of the middle of the cell, but apparently not in a constant position with reference to planes passing through the cilia and contractile vacuoles, as is the rule in *Pteromonas* and in many species of *Chlamydomonas*.

REPRODUCTION. The earliest stages of division found presented the appearance of a cleavage transverse to the longitudinal axis of the cell. More careful consideration, however, here, as in *L. pentagonia*, indicates that an early rotation of the protoplast has eluded observation, for in FIG. 36 two contractile vacuoles, lying in what appears to be the original colorless anterior cytoplasm, now appear on the side of the cell. Even in such a case as that shown in FIG. 37, there is a colorless central region which can only be explained on the supposition that the anterior end of the protoplast had revolved ninety degrees from its original position, here not clockwise as in FIG. 36, but in a vertical plane with reference to the observer. The mother cell retains very much of its original form throughout the process of division, instead of rounding up, which Dangeard describes as being the general rule for *L. Francei*. Here also there may be four or eight daughter cells formed, and they regularly show the

typical obpyriform and lobed shape, and sometimes even show the protoplast somewhat separated from the new cell wall, before escaping from the mother cell. The escape appears to be accomplished by a gradual softening and disintegration of the wall of the mother cell, rather than by rupture at a single point (FIG. 40). The eight daughter cells of this figure are so small as to occasion the surmise that they might be gametes, but conjugation was not seen in this species.

The chief characteristics distinguishing this species from *L. Francei* Dang. are: the general obpyriform shape, the well developed anterior beak or papilla, the lateral pyrenoid, and the persistence of the form of the mother cell during division. The description may be summarized as follows:

Lobomonas rostrata sp. nov. *L.* cellulis vegetativis plus minusve obpiriformibus, rarius ellipsoideis; membrana cellulae in verrucas plures quarum 5–7 vel etiam 10 in facie una apparent producta, atque in polo anteriore rostello cuneiformi seu papilla subduplici instructa, per quod rostellum procurrunt cilia bina longitudine corpus cellulae adaequantia vel longiora, vel breviora: protoplasto primum membranae verrucas complente, deinde plus minus contracto et ellipsoideo: chromatophoro valde excavato, pyrenoidem unum lateralem portante: stigmate bacilliformi, paullulum ante mediam cellulam sito: nucleo majore nunc paene centrali, nunc laterali, rarius posteriori; vacuolis contractilibus binis juxta papillam anteriorem suppositis.

Propagatio fit protoplasto cellulae vegetativae jam immobilis diviso in 4 aut 8 zoosporas, quae formam typicam priusquam evaderunt ex cellulae matricalis membrana adipiscuntur. Copulatio haud observata.

Longit, cell. veg. 5–12 μ; lat. 4–8 μ; long. ciliorum ca. 5–14 μ.

Hab. in aqua pluviali quae colligitur in viis terrenis, et in lacuna quadem lutulenta in pascuo pecuario. Englewood, New Jersey, Sept.–Nov. 1919, Sept. 1920: Shelburne, Vermont, 3 Aug. 1921.

It has been already pointed out elsewhere (6) that *Lobomonas* is to be regarded as a special offshoot from *Chlamydomonas*, not leading to any higher group so far as we know at present. It might be thought simple to derive the genus directly from the Polyblepharidaceae, even from the genus *Dunaliella*, which clearly appears to be the immediate ancestor of *Chlamydomonas*, since it has all the features of cell organization of the latter genus except for the lack of a firm cell wall. When, however, it is recalled that the gametes of *Lobomonas*, reported above for the first time, possess cell walls, it will be more natural to look for

its ancestry among those species of *Chlamydomonas* which posses walled gametes, and are therefore ranked as the primitive members of the genus, since their vegetative cells and gametes differ (visibly) only in point of size. Our two new species of *Lobomonas*, moreover, also resemble a number of the relatively primitive members of *Chlamydomonas* in their method of cell division, namely through a cleavage which is fundamentally longitudinal but early shifts to a transverse position. The question then arises, what influences led to the divergence of cell form, which is practically the sole basis of separation between the species forming the small genus *Lobomonas* and the much larger number comprised in *Chlamydomonas*.

A CONSIDERATION OF MORPHOGENESIS IN PRIMITIVE ALGAE

This whole problem of the origin and inheritance of cell form in primitive organisms is one of very great interest which has received comparatively little attention. Perhaps the most extended discussion of the question has been furnished by D'Arcy Thompson (**22**), who regards surface tension as the paramount factor in the determination of cell form. Though at one point he admits that 'the physical cause of the localized inequalities of surface tension remains unknown', and at other times hints that an internal chemical heterogeneity may have some influence in connection with such differences in surface tension, nevertheless again and again he reiterates his main thesis in regard to unicellular organisms, 'that not only their general configuration but also *their departures from symmetry* may be correlated with the molecular forces manifested in their fluid or semi-fluid surfaces'. This explanation seems to us entirely inadequate and not in harmony with the general weight of evidence. For the particular organisms considered here and in the previous paper on *Brachiomonas* (**6**) we can offer little direct evidence; but there are certain facts which suggest that the conception of the non-homogeneity of the protoplasmic structure of the cell, as developed by Rhumbler (**19**) and Harper (**12**) supplies a much more workable hypothesis than the idea of mere surface tension forces.

Passing over Rhumbler's work on protozoa, the most thorough treatment of morphogenesis within a small group of primitive plants is found in the studies of Harper (**11**, **12**) on *Pediastrum*. He believes that the general four-lobed form

of the cells familiar in most species of *Pediastrum* may well have arisen in evolution as a consequence of the pressure and contact relations of the young cells in the sixteen-celled colony, regarding them merely as surface tension globules: nevertheless he has shown repeatedly that this four-lobed form does not depend in ontogeny upon the forces which may have been responsible for it originally, but that it is inherited and may reach full expression when there is the least possible contact with other cells of the colony. Repeatedly Harper calls attention to the view that though surface tension is commonly acting as a morphogenetic factor, nevertheless 'it is the inherited anomogenous consistency of the cells which is of most significance in determining their form.' In strictly unicellular organisms like *Lobomonas* and *Brachiomonas*, there is an absence of the interaction of contact and pressure stimuli which are important influences in the variation of the *Pediastrum* colony; nevertheless in the fundamental organization of the cell the factors must be parallel in the main. In both cases the lobed form may be regarded as adaptive for the general metabolism of the cell. In *Pediastrum* the development of spines is usually looked upon as a case of primitive differentiation for protection, and in *Lobomonas* and *Brachiomonas* the lobes might easily be conceived of as subserving a similar function: in point of fact, I have observed that when these forms are found in the same pools with smooth-walled ovoid *Chlamydomonas* cells, it is the latter that are devoured by protozoa, while they rarely or never prey upon the lobed forms, even though the latter are smaller. However, when two species of *Chlamydomonas* are present together, sometimes one is rapidly consumed by protozoa while the other is ignored. The anti-selectionist, moreover, might fairly inquire why it is that these genera of bizarre form have produced very few species, in comparison with the extraordinarily successful genus *Chlamydomonas*, which has attained well nigh three score species, so far as taxonomy goes, in recent years.

Wille (**9**, p. 19) ascribed to the zoospores of *Lobomonas* the characteristic of 'deutliche Metabolie.' I cannot find that Dangeard uses this term in connection with this genus; but as defined by him elsewhere* as amoeboid movement, or used

*On désigne sous le nom de métabolie une sorte de mouvement par contraction du corps particulier à quelques Euglènes, Amibes, Monades, etc. C'est ce que nous appelons mouvement amiboïde. [Dangeard: Recherches sur les algues inférieures. Ann. Sci. Nat. Bot. VII. **7**: 144. 1888.]

more exactly by other recent writers (especially in connection with the Polyblepharidaceae) in the sense of euglenoid or amoeboid change of form—and this was the usage of Perty*, by whom the word appears to have been coined in 1852—the term is misleading when applied to *Lobomonas*: or at least it can be used only in a very restricted sense in connection with this genus. The young cells of *Lobomonas* of course do undergo a certain change of outline during the formative process within the wall of the mother cell: ordinarily essentially the mature form is acquired and fixed before their escape, and I have found no evidence that it is appreciably altered afterward during activity, though, in appearance only, the mature cell may be distinctly reminiscent of *Amoeba*. Nevertheless, this idea of amoeboid change of form is most suggestive in a discussion of the formative period of such genera as we have under consideration, and for this restricted period I believe we are entirely justified in drawing a parallel with the results of certain recent researches on the production of pseudopodia.

A brief survey of this work may be useful in this connection. McClendon (**16**) has attempted to explain amoeboid movement as due to local increase in permeability, the *Amoeba* simply receding from the side on which the permeability has been increased. Its author himself admits the difficulty of explaining positive reactions by this theory, and it certainly does not offer any sufficient explanation for the assumption of the characteristic cell-form shown by our developing chlamydomonads. The closely related hypothesis that production of pseudopodia may be accounted for by local variation in surface tension has been widely invoked. The re-statement of this theory lately made by Thompson (**22**) may here be passed over, even as this stimulating writer has all but ignored the evidence which has been accumulating against the surface tension explanation. Both the permeability idea and the surface tension explanation as ordinarily employed are objectionable, in that they depend too

*Metabolie. Hirunter verstehe ich die durch *innere Vorgänge* bedingte wechselnde Gestaltänderung. Seit langem bei den Amiben bekannt ist sie bei den Infusorien so viel als nicht beachtet worden. [Perty: Zur Kenntniss kleinster Lebensformen, p. 127. Berne. 1852.]

Since this term Metabolie seems to be unfamiliar to American botanists because of its very restricted usage, and since it has even been rendered as equivalent to metabolism in a recent German-English dictionary for chemists, it appears to be worth while to call attention to this original definition.

much upon external environmental influences to account for the requisite local variability, though this element is not necessarily involved in either theory.

The beginning of the more recent undermining of the surface tension explanation of form change is found in the work of Jennings (**14**), who from painstaking direct observation reported that the currents in a moving *Amoeba* as a whole 'are not similar to those of a drop of inorganic fluid that is moving or elongating as a result of a local increase or decrease in surface tension', and in particular that 'the movements of material in a forming pseudopodium are not like those in a projection which is produced in a drop of inorganic fluid as a result of a local decrease in surface tension'. From these observations Jennings was forced to the conclusion 'that changes in the surface tension of the body are not the primary factors in the movements and reactions of *Amoeba*'. Similar conclusions from direct observation were announced two years later by Dellinger (**10**), whose clever photographs of *Amoeba* viewed from the side reveal the locomotion of the organism as a sort of 'walking,' rather than a flowing of a fluid substance upon the substratum. These negatory conclusions have been reinforced and extended by a series of recent researches in the field of microdissection (**15**, **21**, **13**). From these there is general agreement that the structure of *Amoeba* (and probably of many other rhizopods and cells of primitive organization) is not of the nature of a simple fluid mass governed chiefly by surface tension forces, but rather that it is a highly non-homogeneous system, consisting of comparatively fluid endoplasm surrounded by an ectoplasm which has often the character of a semi-rigid gel, possessing a considerable elasticity, though the two regions may very probably grade into one another imperceptibly.

Finally, the experiments of Hyman (**13**) reveal in each pseudopodium of *Amoeba* a gradient in susceptibility to potassium cyanide, the susceptibility being greatest at the distal end and decreasing proximally. This susceptibility gradient is regarded as being a metabolic gradient which arises before the pseudopodium appears, 'and hence the metabolic change which produces increased susceptibility is the primary cause of pseudopodium formation.' Liquefaction or solation is regarded as the cause of the extension of a pseudopodium, and coagulation or gelation as the cause of its retraction: the liquefaction is believed to be brought about by the metabolic change just re-

ferred to. This theory that amoeboid movemnt is due to alterations of the colloidal state is only in the nature of confirmation and extension of the view advanced more than forty years ago by Montgomery (**17, 18**) that protoplasmic movement of amoeboid organisms consists in 'an alternate expansion and contraction of organic substance': Montgomery even anticipated Hyman in expressing the idea that the liquefaction which occasions pseudopodium advancement is itself due to metabolic changes, while he anticipated Rhumbler (**19**) in the idea of a non-homogeneity of the primitive protoplasmic mass which permits various functions to be carried on in different regions at the same time.

This somewhat lengthy excursus (which is yet only the briefest possible summary of a voluminous literature) has been introduced here only to bring before botanists, to whom the field may be unfamiliar, facts which it is believed may be directly applied in the case of the chlamydomonads we are considering. The form development of *Lobomonas*, *Brachiomonas*, and *Pteromonas* must be essentially amoeboid for a brief period during the organization of the daughter cells, and we are justified in assuming that their lobes and excrescences are the expression of the same non-homogeneous organization of the protoplast as is characteristic of *Amoeba*.

For this view, furthermore, we may draw an additional parallel from the results of microdissection. In the developing oogonium of *Fucus*, Seifriz reports (**21**) that the viscosity of the protoplasm changes from liquid consistency in the young uninucleate stage to slightly viscous consistency when the division into eight eggs is just complete, and to decidedly viscous consistency (just under the viscosity of glycerine) in the mature normally discharged egg: that is, in Seifriz's scale of ten grades of viscosity—the first attempt on the part of microdissectionists at standardization in this matter—the variation is from grade 3 to grade 6. Yet further, from the behavior of disintegrating eggs of *Fucus*, Seifriz (**20**) finds that the process may be localized in such a manner as to indicate 'a gross structure of the egg plasm, *i. e.*, the protoplasm is composed of many centers of activity in which different chemical reactions take place.' I have recently found a condition almost precisely similar in the case of a newly discovered polyblepharid genus which it is hoped may soon be published. The cells of this species, though surrounded only by an exceedingly delicate protoplasmic membrane, are never-

theless able to maintain for extended periods an elaborately eight-ridged or winged prismatic form, and in disintegration often break only at one or two points, thus permitting the greater body of the protoplasm to remain practically intact, while only small streams ooze out. From these observations we are justified in concluding that such cells as those of *Brachiomonas* and *Lobomonas*, could we apply methods of microdissection, would be found to have protoplasm of a comparatively fluid consistency during division, but that local increase in viscosity gradually permits the fixation of the characteristic lobed form of the cell. When it is recalled that in these two genera the pyrenoids regularly disappear in preparation for cell division, and are reorganized with the maturing of the daughter cells, it will readily be seen that in this reorganization, combined with the ordinary processes involved in division of cells inheriting differentiation of polarity in at least three axes or planes, there is abundant room for the play of such metabolic changes as might well account for considerable differences in viscosity in different parts of the developing daughter protoplasts. It is this non-homogeneity of structure, involving very likely chemical as well as physical differences, which may be regarded as the dominant factor in the determination of form in such organisms. From this standpoint, the problem as to how this characteristic form may be transmitted in heredity does not seem so insoluble as it does on the assumption of form determining unit factors in a germ plasm.

One further point may be emphasized. It is stated by Thompson (**22**) that when 'owing to some heterogeneity of the substance' the operation of uniform surface tension forces is modified so as to result in the production of the ellipsoid cell characteristic of yeast, for example, 'this or any other asymmetrical form, once acquired, may be retained by virtue of the solidification and consequent rigidity of the membranous wall of the cell.' In the case of the organisms with which we are here concerned at least, I am confident, the development of the cell wall is not a necessary condition of the maintenance of specific form; for the new polyblepharid mentioned above, and others of the same group, as well as the gametes of *Brachiomonas*, are able to preserve essentially the same form for long periods, in spite of the fact that they are clothed only with a protoplasmic membrane of such excessive thinness that it is practically undemonstrable. It is then, owing to relative viscosity in their

protoplasmic substance that these motile organisms are able to attain and maintain their specific form.

In both *Brachiomonas* and *Lobomonas* we have noted that not infrequently mature cells exhibit a more or less rounded form of the protoplast inside of the cell wall, while the latter maintains the typical lobed form as fixed in the formative period. Such conditions are not improbably produced by shrinkage due to loss of a certan amount of water from the protoplast, accompanied by a tendency to decrease in viscosity of the outer layer, in which case, surface tension would tend to bring about a more rounded form. In other words, the maturing protoplast might be said to show a tendency to revert to what might be considered the ancestral form in preparation for reproduction.

It is a pleasure to acknowledge my debt to Professor R. A. Harper for numerous stimulating discussions of problems connected with morphogensis, and for reading this paper in manuscript.

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Explanation of plates 5 and 6

Figures drawn with camera lucida from living material kept in hanging drops (VanTieghem cells of standard height of 5 mm.): Leitz compens. oc. 12 was used in combination with oil immers. obj. 1-12 inch, or Spencer 2 mm. The drawings have been reduced one half in reproduction, making the present magnification approximately 1150 diameters for FIGS. 1–21 and 41, and 1375 diameters for FIGS. 22–40.

PLATE 5

LOBOMONAS PENTAGONIA Hazen

FIGS. 1–5. Typical vegetative cells or zoospores: in FIGS. 1–3 the stigma lies on the under side of the cell.

FIG. 5. Anterior polar view: four anterior lobes emtpy, chromatophore filling four posterior lobes.

FIG. 6. Protoplast rotating clockwise in preparation for division; 7 P. M.

FIG. 7. The same cell at 7:05 P. M; beginning of cleavage.

FIG. 8. Division into two zoospores completed at 6:30 P. M.

FIG. 9. Another individual: pyrenoid and cilia beginning to appear in daughter cells; nucleus in most advanced lobe: 10:30 P. M.

FIG. 10. Pentagonal form attained by daughter cells.

FIG. 11. Four daughter cells in one plane.

FIG. 12. Two daughter cells with axes perpendicular to those of the other pair.

FIG. 13. An unusually simple form: cilia contracted into a ball.

FIG. 14. Cell in posterior polar view.

FIGS. 15–20. Stages in conjugation of a pair of slightly unequal gametes: FIG. 15 at 1:45 P. M.; FIG. 16 at 2:15; FIG. 17 at 2:16; FIG. 18 at 2:25; FIG. 19 at 2:40; Fig. 20 at 2:45.

FIG. 21. Zygote formed by conjugation of equal walled gametes.

PLATE 6

LOBOMONAS ROSTRATA Hazen

FIGS. 22–27. Typical young vegetative cells.

FIGS. 28, 29. Mature cells of less common form, but with well developed wall, FIG. 29 showing edge view of beak.

FIG. 30. Anterior polar view.

FIGS. 31, 32. Cells showing persistence of form some time after loss of motility.

FIG. 33. Relatively large mature cell, pyrenoid and stigma underneath.

FIG. 34. The same cell 24 hours later: four daughter cells completely organized except for cilia, 11 P. M.

FIG. 35. One of the four daughter cells, one day later, not very well formed, probably because of unfavorable conditions.

FIG. 36. Beginning of cleavage, after clockwise rotation of protoplast; 9:30 P. M.

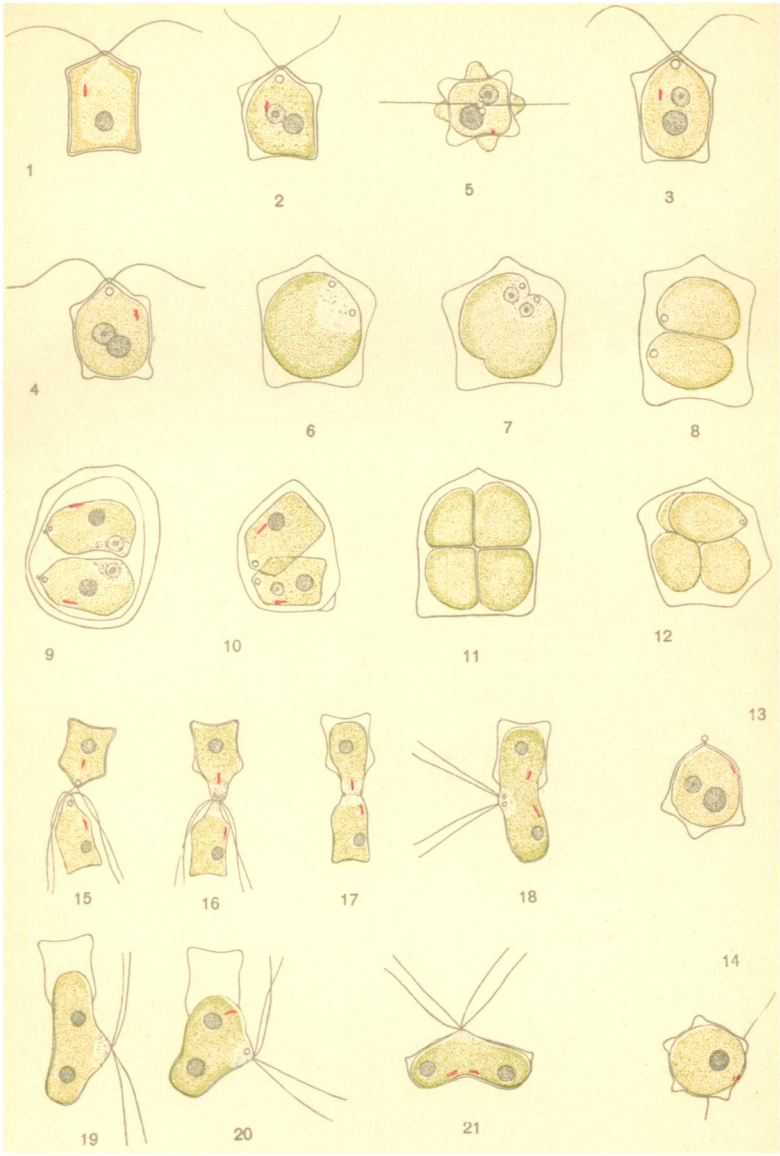
FIG. 37. Similar division, after rotation of protoplast in vertical plane; 10:30 P. M.

FIG. 38. Second cleavage just completed; 10:30 P. M.

FIG. 39. Eight daughter cells (perhaps gametes) 6 P. M.

FIG. 40. The same at 9:30 P. M.: daughter cells held by gelatinized wall of mother cell: cell *a* moved out sluggishly at 8:30; at 10 P. M. cell *b* backed out and presently rested on end, cilia downward as at b^1.

FIG. 41. Mature cell from Shelburne, Vermont, August 3, 1921. All other figures from New Jersey material, in 1919.



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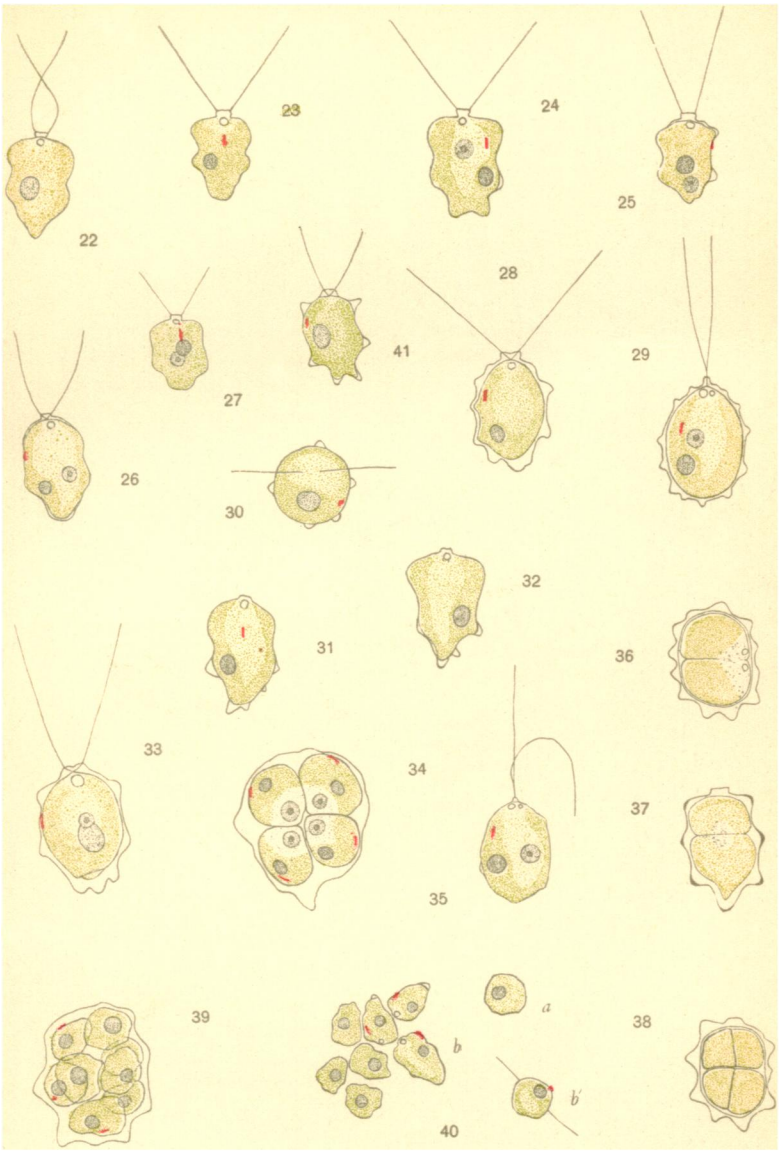

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